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(54) Title: GROWTH DIFFERENTIATION FACTOR-5

(57) Abstract

Growth differentiation factor-5 (GDF-5) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed are diagnostic and therapeutic methods of using the GDF-5 polypeptide and polynucleotide sequences.

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## GROWTH DIFFERENTIATION FACTOR-5

This application is a continuation-in-part application of U.S. Serial No. 08/003,144, filed January 12, 1993.

### BACKGROUND OF THE INVENTION

5        1. *Field of the Invention*

The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF- $\beta$ ) superfamily, which is denoted, growth differentiation factor-5 (GDF-5).

2. *Description of Related Art*

10      The transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer *et al.*, *Nature* 345:167, 1990), *Drosophila* decapentaplegic (DPP) gene product, which is required for dorsal-ventral axis formation and morphogenesis of the imaginal disks (Padgett, *et al.*, *Nature*, 325:81-84, 1987), the *Xenopus* Vg-1 gene product, which localizes to the vegetal pole of eggs (Weeks, *et al.*, *Cell*, 51:861-867, 1987), the activins (Mason, *et al.*, *Biochem. Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation of mesoderm and anterior structures in *Xenopus* embryos (Thomsen *et al.*, *Cell* 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce *de novo* cartilage and bone formation (Sampath, *et al.*, *J. Biol. Chem.* 265:13198, 1990). The TGF- $\beta$ s can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis,

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-2-

hematopoiesis, and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

The proteins of the TGF- $\beta$  family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. For most of the family members that have been studied, the homodimeric species has been found to be biologically active, but for other family members, like the inhibins (Ling, *et al.*, *Nature* 321:779, 1986) and the TGF- $\beta$ s (Cheifetz, *et al.*, *Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

Identification of new factors that are tissue-specific in their expression pattern will provide a greater understanding of that tissue's development and function.

-3-

### SUMMARY OF THE INVENTION

The present invention provides a cell growth and differentiation factor, GDF-5, a polynucleotide sequence which encodes the factor and antibodies which are immunoreactive with the factor. This factor appears to relate to various cell 5 proliferative disorders, especially those involving the uterus, such as endometriosis and uterine tumors, and those involving skeletal tissues.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of uterine origin and which is associated with GDF-5. In another embodiment, the invention provides a method of treating a cell 10 proliferative disorder associated with expression of GDF-5, by suppressing or enhancing GDF-5 activity.

-4-

### BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1A shows expression of GDF-5 mRNA in adult tissues.

FIGURE 1B shows expression of GDF-5 mRNA in embryonic tissues.

FIGURE 2 shows nucleotide and predicted amino acid sequence of GDF-5.

5 The putative tetrabasic processing sites are denoted by stippled boxes.

FIGURE 3A shows the alignment of the C-terminal sequences of GDF-5 with other members of the TGF- $\beta$  family. The conserved cysteine residues are shaded. Dashes denote gaps introduced in order to maximize alignment.

10 FIGURE 3B shows alignment of GDF-5, GDF-6 and GDF-7 C-terminal amino acids.

15 FIGURE 4 shows amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

20 FIGURE 5 shows the expression of GDF-5 in limb mesenchyme of day 12.5 p.c. mouse embryos. Bright field (FIGURE 5a, 5d) and dark field (FIGURE 5b, 5c, 5e, 5f) photomicrographs of transverse (FIGURE 5a-c) and sagittal (FIGURE 5d-f) sections, showing views through forelimb and posterior end of embryo, respectively, after hybridization with  $^{35}$ S-labelled GDF-5 antisense strand (FIGURE 5a,b,d,e) or sense strand control (FIGURE 5c, 5f) probes. Anterior (A), posterior (P), dorsal (D) and ventral (V) orientations are indicated.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides a growth and differentiation factor, GDF-5 and a polynucleotide sequence encoding GDF-5. Unlike other members of the TGF- $\beta$  superfamily, GDF-5 expression is highly tissue specific, being expressed 5 in cells primarily in uterine tissue and skeletal tissue. In one embodiment, the invention provides a method for detection of a cell proliferative disorder of the uterus or skeletal tissue such as bone or cartilage, which is associated with GDF-5 expression. In another embodiment, the invention provides a method for treating a cell proliferative disorder associated with expression of GDF-5 by 10 using an agent which suppresses or enhances GDF-5 activity.

The TGF- $\beta$  superfamily consists of multifunctionally polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory effects, both positive and negative, on other peptide growth factors. The structural homology between the GDF-5 protein of this 15 invention and the members of the TGF- $\beta$  family, indicates that GDF-5 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-5 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

20 The expression of GDF-5 in the uterus suggests a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to contraception, fertility, pregnancy, and cell proliferative diseases. Abnormally low levels of the factor may be indicative of impaired function in the uterus while abnormally high levels may be indicative of hypertrophy, hyperplasia, or 25 the presence of ectopic tissue. Hence, GDF-5 may be useful in detecting not only primary and metastatic neoplasms of uterine origin but in detecting

diseases such as endometriosis as well. In addition, GDF-5 may also be useful as an indicator of developmental anomalies in prenatal screening procedures.

The expression of GDF-5 during embryogenesis and specifically in the precartilaginous mesenchyme associated with early bone formation in the limbs, suggests a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to skeletal development, cartilage differentiation, and cell proliferative diseases. Abnormally low or high levels of GDF-5 may be indicative of various bone dysplasias such as epiphyseal, physeal (growth plate), metaphyseal and diaphyseal hypo- and hyperplasias. Examples of such diseases which may be diagnosed and/or treated using GDF-5 polynucleotides and antibodies include: spondyloepiphyseal dysplasia, dysplasia epiphysialis hemimelica, achondroplasia, metaphyseal dysostosis, hyperchondroplasia, enchondromatosis, hypophosphatasia, osteopetrosis, craniometaphyseal dysplasia, osteogenesis imperfecta, idiopathic osteoporosis, Engelman's disease and hyperphosphatasia (See Harrison's Principles of Internal Medicine, McGraw-Hill Book Co., N.Y., 1987, Chpt. 339).

Several members of the TGF- $\beta$  superfamily possess activities suggesting possible applications for the treatment of cell proliferative disorders, such as cancer. In particular, TGF- $\beta$  has been shown to be potent growth inhibitor for a variety of cell types (Massague, *Cell* 49:437, 1987), MIS has been shown to inhibit the growth of human endometrial carcinoma tumors in nude mice (Donahoe, et al., *Ann. Surg.* 194:472, 1981), and inhibin  $\alpha$  has been shown to suppress the development of tumors both in the ovary and in the testis (Matzuk, et al., *Nature*, 360:313, 1992). GDF-5 may have a similar activity and may therefore be useful as an anti-proliferative agent, such as for the treatment of endometrial cancer or endometriosis.

Many of the members of the TGF- $\beta$  family are also important mediators of tissue repair. TGF- $\beta$  has been shown to have marked effects on the formation of collagen and causes of striking angiogenic response in the newborn mouse (Roberts, et al., *Proc. Natl. Acad. Sci., USA* 83:4167, 1986). The BMP's can 5 induce new bone growth and are effective for the treatment of fractures and other skeletal defects (Glowacki, et al., *Lancet*, 1:959, 1981; Ferguson, et al., *Clin. Orthoped. Relat. Res.*, 227:265, 1988; Johnson, et al., *Clin Orthoped. Relat. Res.*, 230:257, 1988). Sequence homology and expression data together suggest that GDF-5 may have similar activities and may be useful in 10 repair of tissue injury caused by trauma or burns for example.

GDF-5 may play a role in regulation of the menstrual cycle or regulation of uterine function during pregnancy, and therefore, GDF-5, anti-GDF-5 antibodies, or antisense polynucleotides may be useful either in contraceptive regimens, in enhancing the success of *in vitro* fertilization procedures, or in preventing 15 premature labor.

The term "substantially pure" as used herein refers to GDF-5 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-5 using standard techniques for protein purification. The substantially pure polypeptide 20 will yield a single major band on a non-reducing polyacrylamide gel. The purity of the GDF-5 polypeptide can also be determined by amino-terminal amino acid sequence analysis. GDF-5 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-5 remains. Smaller peptides containing the biological activity of GDF-5 are included in the invention.

25 The invention provides polynucleotides encoding the GDF-5 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-5. It is understood that all polynucleotides encoding all or a portion of GDF-5 are

also included herein, as long as they encode a polypeptide with GDF-5 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, GDF-5 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-5 5 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GDF-5 polypeptide encoded 10 by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a cDNA sequence for GDF-5 which is 2329 base pairs in length and contains an open reading frame beginning with a methionine codon at nucleotide 322. The encoded polypeptide is 495 amino acids in length with a molecular weight of about 54.9 K, as determined by 15 nucleotide sequence analysis. The GDF-5 sequence contains a core of hydrophobic amino acids near the N-terminus, suggestive of a signal sequence for secretion. GDF-5 contains one potential N-glycosylation sites at amino acid 183 and two putative tetrabasic proteolytic processing sites RRKRR and KR-at amino acids 371-375 and amino acids 384-385. Cleavage of the precursor at 20 these sites would generate mature C-terminal fragments of 120 or 110 amino acids in length with predicted molecular weights of 13.6K and 12.5K, respectively.

GDF-5 contains all of the highly conserved residues present in other family members, including the seven cysteine residues with their characteristic spacing. Among the known family members, GDF-5 is most highly related to 25 BMP-2 and BMP-4 in the C-terminal portion of the molecule (57% amino acid sequence identity calculated from the first conserved cysteine).

Minor modifications of the recombinant GDF-5 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-5 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the 5 polypeptides produced by these modifications are included herein as long as the biological activity of GDF-5 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For 10 example, one can remove amino or carboxy terminal amino acids which are not required for GDF-5 biological activity.

The nucleotide sequence encoding the GDF-5 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino 15 acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in 20 place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For 25 example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide

-10-

sequences and 2) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-5 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human.

5       Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must 10      be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably 15      performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, 20      for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al.*, *Nucl. Acid Res.*, 9:879, 1981).

25      The development of specific DNA sequences encoding GDF-5 can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a

-11-

eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the 5 least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the 10 entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the 15 formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded 20 DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay et al., *Nucl. Acid Res.* 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly 25 for GDF-5 peptides having at least one epitope, using antibodies specific for GDF-5. Such antibodies can be either polyclonally or monoclonally derived

-12-

and used to detect expression product indicative of the presence of GDF-5 cDNA.

DNA sequences encoding GDF-5 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the GDF-5 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-5 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg et al., *Gene* 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.* 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

-13-

Polynucleotide sequences encoding GDF-5 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art.

5      Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where

10     the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the  $\text{CaCl}_2$  method using procedures well known in the art. Alternatively,  $\text{MgCl}_2$  or  $\text{RbCl}$  can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

15     When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the GDF-5 of the invention, and a second foreign

20     DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

-14-

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

5      The invention includes antibodies immunoreactive with GDF-5 polypeptide or functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, et al., *Nature*, 256:495, 1975). The term antibody as used in this invention is meant to include intact molecules as well as fragments thereof, such as Fab and F(ab')<sub>2</sub>, which are capable of binding an epitopic determinant on GDF-5.

10     The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. The GDF-5 polynucleotide that is an antisense molecule is useful in treating cell proliferative disorders of the various organ systems, particularly, for example, the uterus or skeletal system. Cell proliferative disorders of the skeletal system include those disorders of bone cells and cartilage as described above. Essentially, any disorder involving cells that are normally responsive to GDF-5 could be considered susceptible to treatment with a GDF-5 suppressing reagent.

15     The invention provides a method for detecting a cell proliferative disorder of the uterus or skeletal system (e.g., bone, cartilage) which comprises contacting an anti-GDF-5 antibody with a cell suspected of having a GDF-5 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-5 is labeled with a compound which allows detection of binding to GDF-5.

-15-

For purposes of the invention, an antibody specific for GDF-5 polypeptide may be used to detect the level of GDF-5 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is tissue of uterine origin, specifically endometrial tissue or skeletal tissue such as bone and cartilage. The level of GDF-5 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-5-associated cell proliferative disorder. 5 Preferably the subject is human.

The antibodies of the invention can be used in any subject in which it is 10 desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize 15 antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous 20 modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The antibodies of the invention can be bound to many different carriers and 25 used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled

in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in 5 the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

10 Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific anti- 15 haptens antibodies.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to 20 enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable 25 that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for *in vivo* diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are <sup>111</sup>In, <sup>97</sup>Ru, <sup>67</sup>Ga, <sup>68</sup>Ga, <sup>72</sup>As, <sup>89</sup>Zr, and <sup>201</sup>Tl.

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and

-18-

paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include <sup>157</sup>Gd, <sup>55</sup>Mn, <sup>162</sup>Dy, <sup>52</sup>Cr, and <sup>56</sup>Fe.

The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-5-associated disease in a subject. 5 Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids and tissues, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-5-associated disease is effective. The term 10 "ameliorate" denotes a lessening of the detrimental effect of the GDF-5-associated disease in the subject receiving therapy.

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed 15 to this sequence. Thus, where a cell-proliferative disorder is associated with the expression of GDF-5, nucleic acid sequences that interfere with GDF-5 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific 20 GDF-5 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense 25 nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely

to cause problems than larger molecules when introduced into the target GDF-5-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

5        Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J.Amer.Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because 10      they are sequence-specific, only mRNAs with particular sequences are inactivated.

15      There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a 20      specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

25      The present invention also provides gene therapy for the treatment of cell proliferative disorders which are mediated by GDF-5 protein. Such therapy would achieve its therapeutic effect by introduction of the GDF-5 antisense polynucleotide into cells having the proliferative disorder. Delivery of antisense GDF-5 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred

-20-

for therapeutic delivery of antisense sequences is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-5 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the GDF-5 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal

include, but are not limited to  $\psi$ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be 5 packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag*, *pol*, and *env*, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells 10 release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-5 antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The 15 preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0  $\mu$ m can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions 20 can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be 25 present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4)

accurate and effective expression of genetic information (Mannino, *et al.*, *Biotechniques*, 5:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in 5 combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and 10 gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

15 The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to 20 distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and 25 cell types other than the naturally occurring sites of localization.

-23-

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking 5 groups can be used for joining the lipid chains to the targeting ligand.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

EXAMPLE 1

10 IDENTIFICATION AND ISOLATION OF A NOVEL  
TGF- $\beta$  FAMILY MEMBER

To identify a new member of the TGF- $\beta$  superfamily, degenerate oligonucleotides were designed which corresponded to two conserved regions among the known family members: one region spanning the two tryptophan residues 15 conserved in all family members except MIS and the other region spanning the invariant cysteine residues near the C-terminus. These primers were used for polymerase chain reactions on mouse genomic DNA followed by subcloning the PCR products using restriction sites placed at the 5' ends of the primers, picking individual *E. coli* colonies carrying these subcloned inserts, and using 20 a combination of random sequencing and hybridization analysis to eliminate known members of the superfamily.

GDF-5 was identified by polymerase chain reaction (PCR) using mouse genomic DNA with the following primers:

SJL136:5'-CCGGAATTCGG(G/A/T/C)TGGGA(G/A)(A/C)G(G/A/T/C)TGG(G/A)T

25 (G/A/T/C)(G/A)T-3' (SEQUENCE ID NO. 1)

-24-

SJL121:5'-CCGGAATTC(G/A)CAICC(G/A)CA(T/C)TC(G/A)TCIACIACCAT(G/A)  
TC(T/C)TC(G/A)TA-3' (SEQUENCE ID NO. 2)

5 SJL 136 corresponds to the amino acid sequence GWE(R/S)W(V/I/M)(V/I/M),  
(SEQUENCE ID NO. 3) and the complement of SJL 121 corresponds to the  
amino acid sequence YEDMVVDECGC (SEQUENCE ID NO. 4). Both  
oligonucleotide sets were designed to contain an EcoRI restriction site at the  
5'end to facilitate subcloning. PCR was carried out for 40 cycles at 94°C for  
1', 50°C for 2' and 72°C for 3.5'.

10 Human GDF-5 was isolated by PCR using human genomic DNA with the  
following primers:

SJL 141: 5'-CCGGAATTGGTGG(G/C/A)A(G/A/T/C)(A/G)A(T/C)TGG(A/G)  
T(A/G)T(T/G)CICC-3' (SEQUENCE ID NO. 5)

SJL145:5'-CCGGAATTC(G/A)CAI(G/C)C(G/A)CAIG(C/A)(G/A/T/C)TCIACI(G/A)  
(T/C)CAT-3' (SEQUENCE ID NO. 6)

15 SJL 141 corresponds to the amino acid sequence GW(H/Q/N/K/D/E)(D/N)W-  
(V/I/M)(V/I/M)(A/S)P (SEQUENCE ID NO. 7) and the complement of SJL 145  
corresponds to the amino acid sequence M(V/I/M/T/A)V(D/E)(A/S)C(G/A)C  
(SEQUENCE ID NO. 8). Both the oligonucleotide sets were designed to  
contain an EcoRI restriction site at the 5' end to facilitate subcloning. PCR was  
20 carried out for 40 cycles at 94°C for 1 min., 50°C for 2 min., and 72°C for 2  
min. Partial sequence analysis of the human PCR product revealed no  
predicted amino acid differences between mouse and human GDF-5.

25 PCR products of approximately 280 bp were gel-purified, digested with Eco RI,  
gel-purified again, and subcloned in the Bluescript vector (Stratagene, San  
Diego, CA). Bacterial colonies carrying individual subclones were picked into  
96 well microtiter plates, and multiple replicas were prepared by plating the  
cells onto nitrocellulose. The replicate filters were hybridized to probes

-25-

representing known members of the family, and DNA was prepared from non-hybridizing colonies for sequence analysis.

RNA isolation and Northern analysis were carried out as described previously (Lee, S.J., *Mol. Endocrinol.*, **4**:1034, 1990). An oligo dT-primed cDNA library 5 was prepared from 2.5-3 µg of 12.5 day gestation CD-1 mouse embryo poly A-selected RNA in the lambda ZAP II vector according to the instructions provided by Stratagene. The library was amplified prior to screening. Filters were hybridized as described previously (Lee, S.-J., *Proc. Natl. Acad. Sci. USA*, **88**:4250-4254, 1991). DNA sequencing of both strands was carried out 10 using the dideoxy chain termination method (Sanger, et al., *Proc. Natl. Acad. Sci., USA* **74**:5463-5467, 1977) and a combination of the S1 nuclease-/exonuclease III strategy (Henikoff, S., *Gene*, **28**:351-359, 1984) and synthetic oligonucleotide primers.

#### EXAMPLE 2

#### EXPRESSION PATTERN AND SEQUENCE OF GDF-5

15 To determine the expression pattern of GDF-5, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. RNA isolation and Northern analysis were carried out as described previously (Lee, S.J., *Mol. Endocrinol.*, **4**:1034, 1990). Five micrograms of twice polyA-selected RNA prepared from each tissue were electrophoresed on formaldehyde gels, blotted 20 and probed with GDF-5. As shown in Figure 1A, the GDF-5 probe detected an approximately 2.5 kb mRNA expressed primarily in the uterus and at lower levels in other adult tissues in the mouse, including placenta, brain, thymus, lung, kidney, and adrenal gland. The GDF-5 probe also detected a larger 25 mRNA in the oviduct. High levels of GDF-5 transcripts were also detected in mouse embryos, particularly at day 12.5 of gestation (FIGURE 1B).

A CD-1 day 12.5 whole mouse embryo cDNA library was constructed in lambda ZAP II and screened with a probe derived from the GDF-5 PCR product. The nucleotide sequence of the longest hybridizing clone is shown in Figure 2. The in-frame termination codons upstream of the putative initiating ATG and the consensus polyadenylation signals are underlined. The poly A tails are not shown. Numbers indicate nucleotide position relative to the 5' end. The 2329 bp sequence contains a long open reading frame beginning with a methionine codon at nucleotide 322 and potentially encoding a protein 495 amino acids in length with a molecular weight of 54.9 K. Like other TGF- $\beta$  family members, the GDF-5 sequence contains a core of hydrophobic amino acids near the N-terminus suggestive of a signal sequence for secretion. GDF-5 contains a single potential N-glycosylation sites at asparagine residue 183 (denoted by the plain box) and two putative tetrabasic proteolytic processing sites at amino acids 371-375 (denoted by the stippled box) and amino acids 384-385. GDF-5 contains all of the highly conserved residues present in other family members (Figures 3 and 4), including the seven cysteine residues with their characteristic spacing. Among the known mammalian family members, GDF-5 is most highly related to BMP-2 and BMP-4 in the C-terminal portion of the molecule (57% amino acid sequence identity calculated from the first 20 conserved cysteine).

Although the C-terminal portion of GDF-5 clearly shows homology with the other family members, the sequence of GDF-5 is significantly diverged from those of the other family members (Figures 3 and 4). Figure 3 shows the alignment of the C-terminal sequences of GDF-5 with the corresponding regions of human GDF-1 (Lee, *Proc. Natl. Acad. Sci. USA* 88:4250-4254, 1991), human Vgr-1 (Celeste, et al., *Proc. Natl. Acad. Sci. USA* 87:9843-9847, 1990), human OP-1 (Ozkaynak, et al., *EMBO J.* 9:2085-2093, 1990), human BMP-5 (Celeste, et al., *Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human BMP-3 (Wozney, et al., *Science*, 242:1528-1534, 1988), human MIS (Cate, et al. *Cell*,

-27-

45:685-698, 1986), human inhibin  $\alpha$ ,  $\beta$ A, and  $\beta$ B (Mason, et al., *Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), human TGF- $\beta$ 1 (Deryck, et al., *Nature*, 316:701-705, 1985), human TGF- $\beta$ 2 (deMartin, et al., *EMBO J.*, 6:3673-3677, 1987), human TGF- $\beta$ 3 (ten Dijke, et al., *Proc. Natl. Acad. Sci. USA*, 85:4715-4719, 1988), chicken TGF- $\beta$ 4 (Jakowlew, et al., *Mol. Endocrinol.* 2:1186-1195, 1988), and *Xenopus* TGF- $\beta$ 5 (Kondaiah, et al., *J. Biol. Chem.* 265:1089-1093, 1990). The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize the alignment.

10 Figure 4 shows the amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

15 The degree of sequence identify with known family members ranges from a minimum of 24% with inhibin alpha to a maximum of 57% with BMP-2 and BMP-4. GDF-5 shows no significant sequence homology to other family members in the pro-region of the molecule.

### EXAMPLE 3

20 The results in Example 2 show that during the development of the mouse embryo, the expression of GDF-5 begins at approximately day 10.5 post coitum (p.c.) and peaks at day 12.5 p.c., as indicated by the presence of a 2.5 kilobase (kb) major transcript (FIGURE 1B). Of the adult mouse tissues examined, uterus contained the highest level of the 2.5 kb transcript, while low levels were detected in placenta (day 10.5 p.c.), oviduct, brain, thymus, heart, 25 lung, kidney and adrenal gland (FIGURE 1A). In oviduct tissue, the GDF-5

-28-

probe also detected a larger transcript of approximately 3.6 kb. GDF-5 transcripts were also detected by Northern blot analysis in femur and calvaria of newborn mice.

5 In order to characterize in more detail, the expression of GDF-5 in embryonic tissues,  $^{35}$ S-labelled probes synthesized from a portion of the cDNA clone encoding the relatively nonconserved prepro-region were hybridized *in situ* to serial sections of day 12.5 p.c. embryos. Day 12.5 p.c. female CD-1 mouse embryos were fixed and embedded in paraffin as described (Jones, C.M., et al., *Development*, 111:531-542, 1991).  $^{35}$ S-labelled antisense or sense strand RNA probes were synthesized by *in vitro* transcription from a template containing nucleotides 308 through 1446 of the GDF-5 cDNA clone (FIGURE 2). Eight micron sections were hybridized with antisense or sense strand probe at  $4 \times 10^5$  counts per minute/ $\mu$ l essentially as described (Jones, C.M., et al., *supra*) except that the proteinase K and acetic anhydride treatments were omitted, washes in 50% formamide, 2 x SSC, 0.1 M DTT were carried out at 65°C, and the final wash in 0.1 x SSC was carried out at 37°C. Slides were developed after a 4-6 week exposure time with Kodak NTB3 emulsion and were stained with hematoxylin and eosin.

20 FIGURE 5 shows shows the expression of GDF-5 in limb mesenchyme of day 12.5 p.c. mouse embryos. Bright field (FIGURE 5a, 5d) and dark field (FIGURE 5b, 5c, 5e, 5f) photomicrographs of transverse (FIGURE 5a-c) and sagittal (FIGURE 5d-f) sections, showing views through forelimb and posterior end of embryo, respectively, after hybridization with  $^{35}$ S-labelled GDF-5 antisense strand (FIGURE 5a,b,d,e) or sense strand control (FIGURE 5c, 5f) probes. 25 Serial sections revealed hybridization to be localized to proximal (closed arrows) and distal (open arrows) mesenchyme in the forelimb (FIGURE 5a-c) and hindlimb (FIGURE 5d-f). Anterior (A), posterior (P), dorsal (D) and ventral (V) orientations are indicated.

GDF-5 transcripts were detected in both proximal and distal precartilaginous mesenchyme of the forelimbs and hindlimbs (FIGURE 5). No other major sites of hybridization in the embryo were detected. The development of the long bones of the limbs begins with the condensation of mesenchyme, which 5 differentiates into cartilage-forming cells. Osteogenic cells eventually invade the cartilage matrix and produce a bone matrix which becomes ossified (Rosen, V., et al., *Trends Genet.*, 8:97-102, 1992). In the mouse embryo at 12.5 days p.c., cartilage formation is just beginning in the long bones, and no sign of 10 ossification is yet seen (Kaufman, M.H., *The Atlas of Mouse Development*, Academic Press, Inc., 1992). The peak of GDF-5 expression at this stage (FIGURE 1B) and its primary location in the precartilaginous limb mesenchyme suggest that GDF-5 may affect the production, proliferation, and/or differentiation of the mesenchyme cells.

In addition to GDF-5, two other members of the TGF- $\beta$  superfamily have been 15 suggested to play a role in limb development. In particular, BMP-2 and BMP-4 are known to be expressed in the apical ectodermal ridge (AER) during mid-gestation at day 10.5 p.c. (Lyons, K.M., et al., *Development*, 109:833-844, 1990; Jones, C.M., et al., *Development*, 111:531-542, 1991). BMP-2 has been shown to inhibit the proliferation of mesenchyme cells in cultured limbs of mid-gestational embryos from which the AER had been removed (Niswander, L., et al., *Nature*, 361:68-71, 1993). Because BMP-2 and BMP-4 are also known 20 to be expressed in limb mesenchyme at day 12.5 p.c. and because the active form of growth factors in this family is generally a disulfied-linked dimer, the possibility exists that homodimers or heterodimers of GDF-5, BMP-2 and BMP-4 25 may have distinct roles in limb development.

So far, the only bone morphogenetic protein for which mutants have been found is BMP-5, encoded by the mouse short ear locus (Kingsley, D.M., et al., *Cell*, 71:399-419, 1992). Mice homozygous for the short ear mutation, which

-30-

causes a range of skeletal defects, have alterations in the size and shape of precartilaginous condensations of mesenchyme (Green, E.L., et al., *J. Morphol.*, 70:1-19, 1942). Skeletal defects of the limbs and digits may be caused by mutations in the mouse gene encoding GDF-5. Like BMP-5, GDF-5 controls 5 particular aspects of skeletal morphology during development.

-31-

-- SUMMARY OF SEQUENCES

SEQUENCE ID NO 1 is the nucleotide sequence for the GDF-5 primer, SJL136.  
SEQUENCE ID NO 2 is the nucleotide sequence for the GDF-5 primer, SJL121.  
SEQUENCE ID NO 3 is the amino acid sequence for the GDF-5 primer,  
5 SJL136.  
SEQUENCE ID NO 4 is the amino acid sequence for the GDF-5 primer,  
SJL121.  
SEQUENCE ID NO 5 is the nucleotide sequence for the GDF-5 primer, SJL141.  
SEQUENCE ID NO 6 is the nucleotide sequence for the GDF-5 primer, SJL145.  
10 SEQUENCE ID NO 7 is the amino acid sequence for the GDF-5 primer,  
SJL141.  
SEQUENCE ID NO 8 is the amino acid sequence for the GDF-5 primer,  
SJL145.  
SEQUENCE ID NO 9 is the nucleotide and deduced amino acid sequence for  
15 GDF-5.  
SEQUENCE ID NO 10 is the deduced amino acid sequence for GDF-5.  
SEQUENCE ID NO 11 is the amino acid sequence for GDF-1.  
SEQUENCE ID NO 12 is the amino acid sequence for GDF-3.  
SEQUENCE ID NO 13 is the amino acid sequence for GDF-5.  
20 SEQUENCE ID NO 14 is the amino acid sequence for GDF-9.  
SEQUENCE ID NO 15 is the amino acid sequence for BMP-2.  
SEQUENCE ID NO 16 is the amino acid sequence for GDF-4.  
SEQUENCE ID NO 17 is the amino acid sequence for Vgr-1.  
SEQUENCE ID NO 18 is the amino acid sequence for Op-1.  
25 SEQUENCE ID NO 19 is the amino acid sequence for BMP-5.  
SEQUENCE ID NO 20 is the amino acid sequence for BMP-3.  
SEQUENCE ID NO 21 is the amino acid sequence for MIS.  
SEQUENCE ID NO 22 is the amino acid sequence for inhibin- $\alpha$ .  
SEQUENCE ID NO 23 is the amino acid sequence for inhibin- $\beta\alpha$ .

-32-

SEQUENCE ID NO 24 is the amino acid sequence for inhibin- $\beta\beta$ .

SEQUENCE ID NO 25 is the amino acid sequence for TGF- $\beta$ 1.

SEQUENCE ID NO 26 is the amino acid sequence for TGF- $\beta$ 2.

SEQUENCE ID NO 27 is the amino acid sequence for TGF- $\beta$ 3.

-33-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SE-JIN LEE  
HUYNH, THANH

5 (ii) TITLE OF INVENTION: GROWTH DIFFERENTIATION FACTOR-5

(iii) NUMBER OF SEQUENCES: 27

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(C) CITY: LOS ANGELES  
(D) STATE: CALIFORNIA  
(E) COUNTRY: US  
(F) ZIP: 90067

15 (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

20 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT  
(B) FILING DATE: 1/12/94  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

25 (A) NAME: WETHERELL, JR. PH.D., JOHN R.  
(B) REGISTRATION NUMBER: 31,678  
(C) REFERENCE/DOCKET NUMBER: FD3256 CIP OF PD2280

(ix) TELECOMMUNICATION INFORMATION:

30 (A) TELEPHONE: 619/455-5100  
(B) TELEFAX: 619-455-5110

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 28 base pairs  
(B) TYPE: nucleic acid

-34-

- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- 5 (B) CLONE: 136

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10 CCGGAATTCTG GNTGGGARMG NTGGRTNR

28

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 base pairs
- (B) TYPE: nucleic acid
- 15 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 121

20 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..42
- (D) OTHER INFORMATION: /note- "WHERE "B" OCCURS, B -  
INOSINE"

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCTG CABCCRCAYT CRTCBACBAC CATRTCYTCR TA

42

(2) INFORMATION FOR SEQ ID NO:3:

-35-

(i) SEQUENCE CHARACTERISTICS:  
5 (A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:  
10 (B) CLONE: 136

10 (ix) FEATURE:  
(A) NAME/KEY: Peptide  
(B) LOCATION: 1..7  
(D) OTHER INFORMATION: /note- "R = Arg, Ser; V = Val,  
Ileu, Met."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

15 Gly Trp Glu Arg Trp Val Val  
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
20 (A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:  
25 (B) CLONE: 121

(ix) FEATURE:  
(A) NAME/KEY: Peptide  
(B) LOCATION: 1..11

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

-36-

Tyr Glu Asp Met Val Val Asp Glu Cys Gly Cys  
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 35 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10 (vii) IMMEDIATE SOURCE:

(A) LIBRARY: 141

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 1..35  
15 (D) OTHER INFORMATION: /note- "WHERE "B" OCCURS, B -  
INOSINE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCGGAATTCTG GBTGGVANRA YTGGRTBRTB KCBCC

35

(2) INFORMATION FOR SEQ ID NO:6:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:  
(B) CLONE: 145

(ix) FEATURE:

(A) NAME/KEY: CDS  
30 (B) LOCATION: 1..33

-37-

(D) OTHER INFORMATION: /note- "WHERE "B" OCCURS, B -  
INOSINE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCGGAATTCR CABSCRCABG MNTCBACBRY CAT

33

5 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 141

15 (ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..9
- (D) OTHER INFORMATION: /note- "H - His, Gln, Asn, Lys,  
Glu, Asp; D - Asp, Asn; V - Val, Ile, Met; A -  
Glu, Ser. "

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Trp His Asp Trp Val Val Ala Pro  
1 5

(2) INFORMATION FOR SEQ ID NO:8:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

-38-

(vii) IMMEDIATE SOURCE:

(B) CLONE: 145

(ix) FEATURE:

(A) NAME/KEY: Peptide

5 (B) LOCATION: 1..8

(D) OTHER INFORMATION: /note- "V - Val, Ile, Met, Thr,  
Ala; D - Asp, Glu; A - Ala, Ser; G - Gly, ..."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

10 Met Val Val Asp Ala Cys Gly Cys  
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2329 base pairs

(B) TYPE: nucleic acid

15 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: GDF-5

20 (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 322..1807

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTCAAGCCCT CAGTCAGTTG TGCAGGAGAA AGGGGGCGGT CGGCTTTCTC CTTTCAAGAA	60
25 CGAGTTATTT TCAGCTGCTG ACTGGAGACG GTGCACGTCT GGACACGGGA GCACTTCCAC	120
TATGGGACTG GATACAGACA CACGCCGGC GGACTTCAAG ACACTCAGAC TGAGGAGAAA	180
GCCCTGCCTG CTGCTGCTGC TGCTGCTGCT GCCACCGCTG CCTCTGAAGA CCCACTCCTT	240
TCATGGTTTT TCCTGCCAAG CCAGAGGCAC CTTCGCTGCT ACGGCCTTC TCTGTGGTGT	300

-39-

	CATTCAGCGG CTGGCCAGAG G ATG AGA CTC CCC AAA CTC CTC ACT CTT TTG	351
	Met Arg Leu Pro Lys Leu Leu Thr Leu Leu	
	1 5 10	
5	CTG TGG CAC CTG GCT TGG CTG GAC CTG GAA CTC ATC TGC ACT GTG CTG	399
	Leu Trp His Leu Ala Trp Leu Asp Leu Glu Leu Ile Cys Thr Val Leu	
	15 20 25	
	GGT GCC CCT GAC TTA GGA CAG AGA ACC CCA GGG GCC AAG CCA GGG TTG	447
	Gly Ala Pro Asp Leu Gly Gln Arg Thr Pro Gly Ala Lys Pro Gly Leu	
	30 35 40	
10	ACC AAA GCG GAG GCC AAG GAG AGG CCA CCC CTG GCC AGG AAT GTC TTT	495
	Thr Lys Ala Glu Ala Lys Glu Arg Pro Pro Leu Ala Arg Asn Val Phe	
	45 50 55	
15	AGG CCA GGG GGT CAT ATC TAT GGT GTG GGG GCC ACC AAT GCC AGG GCC	543
	Arg Pro Gly Gly His Ile Tyr Gly Val Gly Ala Thr Asn Ala Arg Ala	
	60 65 70	
20	AAG GGA AGC TCT GGG CAG ACA CAG GCC AAG AAG GAT GAA CCC AGA AAG	591
	Lys Gly Ser Ser Gly Gln Thr Gln Ala Lys Lys Asp Glu Pro Arg Lys	
	75 80 85 90	
25	ATG CCC CCC AGA TCC GGT GGC TCT GAA ACC AAG CCA GGA CCC TCT TCC	639
	Met Pro Pro Arg Ser Gly Ser Glu Thr Lys Pro Gly Pro Ser Ser	
	95 100 105	
	CAG ACT AGA CAG GCT GCA GCC CGG ACT GTA ACC CCA AAA GGA CAG CTT	687
	Gln Thr Arg Gln Ala Ala Ala Arg Thr Val Thr Pro Lys Gly Gln Leu	
	110 115 120	
30	CCT GGG GGC AAA GCA TCT TCA AAA GCA GGA TCT GCC CCC AGC TCC TTC	735
	Pro Gly Gly Lys Ala Ser Ser Lys Ala Gly Ser Ala Pro Ser Ser Phe	
	125 130 135	
	CTG CTG AAG AAG ACC AGG GAG CCT GGG ACC CCT CGA GAG CCC AAG GAG	783
	Leu Leu Lys Lys Thr Arg Glu Pro Gly Thr Pro Arg Glu Pro Lys Glu	
	140 145 150	
	CCG TTC CGC CCG CCC CCC ATC ACA CCC CAC GAA TAC ATG CTC TCC CTG	831
	Pro Phe Arg Pro Pro Pro Ile Thr Pro His Glu Tyr Met Leu Ser Leu	
	155 160 165 170	
	TAC AGG ACG CTG TCC GAT GCT GAC AGA AAG GGA CCT AAC AGC AGC GTG	879

-40-

	Tyr Arg Thr Leu Ser Asp Ala Asp Arg Lys Gly Gly Asn Ser Ser Val			
	175	180	185	
	AAG TTG GAG GCT GGC CTG GCC AAC ACC ATC ACC AGC TTT ATT GAC AAA			927
5	Lys Leu Glu Ala Gly Leu Ala Asn Thr Ile Thr Ser Phe Ile Asp Lys	190	195	200
	GGG CAA GAT GAC CGA GGC CCT GCG GTC AGG AAG CAG AGG TAC GTG TTT			975
	Gly Gln Asp Asp Arg Gly Pro Ala Val Arg Lys Gln Arg Tyr Val Phe	205	210	215
10	GAC ATC AGT GCC TTG GAG AAG GAT GGG CTG TTG GGG GCT GAA CTG CGG			1023
	Asp Ile Ser Ala Leu Glu Lys Asp Gly Leu Leu Gly Ala Glu Leu Arg	220	225	230
	ATC TTA CGG AAG AAG CCC TTG GAC GTG GCC AAG CCA GCG GTC CCC AGT			1071
	Ile Leu Arg Lys Lys Pro Leu Asp Val Ala Lys Pro Ala Val Pro Ser	235	240	245
15	AGC GGG CGG GTT GCC CAA CTG AAG CTG TCC AGC TGC CCC AGC GGC CGG			1119
	Ser Gly Arg Val Ala Gln Leu Lys Leu Ser Ser Cys Pro Ser Gly Arg	255	260	265
20	CAG CCG GCA GCC TTG CTG GAT GTG CGC TCC GTG CCA GGC CTG GAT GGA			1167
	Gln Pro Ala Ala Leu Leu Asp Val Arg Ser Val Pro Gly Leu Asp Gly	270	275	280
	TCT GGC TGG GAG GTG TTC GAC ATC TGG AAG CTC TTC CGA AAT TTT AAG			1215
	Ser Gly Trp Glu Val Phe Asp Ile Trp Lys Leu Phe Arg Asn Phe Lys	285	290	295
25	AAC TCA GCG CAG CTG TGC CTG GAG CTG GAG GCC TGG GAA CGG CGG CGG			1263
	Asn Ser Ala Gln Leu Cys Leu Glu Leu Glu Ala Trp Glu Arg Gly Arg	300	305	310
	GCC GTG GAC CTC CGT GGC CTG GGC TTT GAA CGC ACT GCC CGA CAG GTC			1311
	Ala Val Asp Leu Arg Gly Leu Gly Phe Glu Arg Thr Ala Arg Gln Val	315	320	325
30	CAC GAG AAA GCC TTG TTC CTA GTG TTT GGT CGT ACC AAG AAA CGG GAC			1359
	His Glu Lys Ala Leu Phe Leu Val Phe Gly Arg Thr Lys Lys Arg Asp	335	340	345

-41-

	CTG TTC TTT AAT GAG ATT AAG GCC CGC TCT GGC CAG GAT GAC AAG ACT Leu Phe Phe Asn Glu Ile Lys Ala Arg Ser Gly Gln Asp Asp Lys Thr 350 355 360	1407
5	GTG TAT GAA TAT TTG TTC AGC CAG CGG CGG AAA CGC CGG GCC CCA TTG Val Tyr Glu Tyr Leu Phe Ser Gln Arg Arg Lys Arg Arg Ala Pro Leu 365 370 375	1455
	GCC AAT CGC CAG GGC AAG CGA CCC AGC AAG AAC CTC AAG GCT CGC TGC Ala Asn Arg Gln Gly Lys Arg Pro Ser Lys Asn Leu Lys Ala Arg Cys 380 385 390	1503
10	AGT CGC AAG GCC TTG CAT GTC AAC TTC AAG GAC ATG GGC TGG GAC GAC Ser Arg Lys Ala Leu His Val Asn Phe Lys Asp Met Gly Trp Asp Asp 395 400 405 410	1551
15	TGG ATC ATC GCA CCT CTT GAG TAT GAG GCC TTC CAC TGC GAA GGA CTG Trp Ile Ile Ala Pro Leu Glu Tyr Glu Ala Phe His Cys Glu Gly Leu 415 420 425	1599
	TGT GAG TTC CCC TTG CGC TCC CAC TTG GAG CCC ACA AAC CAC GCA GTC Cys Glu Phe Pro Leu Arg Ser His Leu Glu Pro Thr Asn His Ala Val 430 435 440	1647
20	ATT CAG ACC CTA ATG AAC TCT ATG GAC CCT GAA TCC ACA CCA CCC ACT Ile Gln Thr Leu Met Asn Ser Met Asp Pro Glu Ser Thr Pro Pro Thr 445 450 455	1695
	TGT TGT GTG CCT ACA CGG CTG AGT CCT ATT AGC ATC CTC TTC ATC GAC Cys Cys Val Pro Thr Arg Leu Ser Pro Ile Ser Ile Leu Phe Ile Asp 460 465 470	1743
25	TCT GCC AAC AAC GTG GTG TAT AAA CAG TAC GAG GAC ATG GTC GTG GAA Ser Ala Asn Asn Val Val Tyr Lys Gln Tyr Glu Asp Met Val Val Glu 475 480 485 490	1791
	TCT TGT GGC TGC AGG T AGCAGCACCG GCCCACCTGT CTTCCAGGGT GGCACATCCA Ser Cys Gly Cys Arg 495	1847
30	GAGACTACCC CCTCTACAGG TTCCTGGAGT AACAGAGAGC CTGTGAAGCT GCTGCCGAA GTTTCCTGGC AGCCTGCAGG AAAGAGTTCT CAGCAGGGCTT ACTCTCTGGA TGTGATCTGG ACTAAAGAGA TCACCTCTG AAGATTCTG CCCAAGGAAC AGACTCTGAG TGGGCCTGGG	1907 1967 2027

-42-

	GCTCAGGAAA GGTGTTCTTA ATGAGATTCA GTTCACCATC TCTCCTGCCG GGGCCGGAGA	2087
	CCTTCATTTC TCTCCAGACT CTCCAGAGAA GTTGTAGCTA TATCCTAAGC TCTTTAAGGG	2147
	AGAGCTGTCT CCTCCCTTGAA TCACCTTTGT GCCTGGTGAC TTTCTGCCAC GAGATGTTCA	2207
	TTACAGGGGC TGGGCAAAGA AGGGGAAAGG GCTTGGGCAG GGGTGAAGAG AAGAGTATGA	2267
5	GCCTAATTAG ACTGTTAGAT TAAAATGTAC ATCGATGACA TAAAAGCTGA ATCTTCATGG	2327
	CT	2329

## (2) INFORMATION FOR SEQ ID NO:10:

	(i) SEQUENCE CHARACTERISTICS:		
10	(A) LENGTH: 495 amino acids		
	(B) TYPE: amino acid		
	(D) TOPOLOGY: linear		
	(ii) MOLECULE TYPE: protein		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:		
15	Met Arg Leu Pro Lys Leu Leu Thr Leu Leu Leu Trp His Leu Ala Trp		
	1	5	10
	Leu Asp Leu Glu Leu Ile Cys Thr Val Leu Gly Ala Pro Asp Leu Gly		
	20	25	30
	Gln Arg Thr Pro Gly Ala Lys Pro Gly Leu Thr Lys Ala Glu Ala Lys		
	35	40	45
20	Glu Arg Pro Pro Leu Ala Arg Asn Val Phe Arg Pro Gly Gly His Ile		
	50	55	60
	Tyr Gly Val Gly Ala Thr Asn Ala Arg Ala Lys Gly Ser Ser Gly Gln		
	65	70	75
	80		
25	Thr Gln Ala Lys Lys Asp Glu Pro Arg Lys Met Pro Pro Arg Ser Gly		
	85	90	95
	Gly Ser Glu Thr Lys Pro Gly Pro Ser Ser Gln Thr Arg Gln Ala Ala		
	100	105	110

-43-

Ala Arg Thr Val Thr Pro Lys Gly Gln Leu Pro Gly Gly Lys Ala Ser  
115 120 125

Ser Lys Ala Gly Ser Ala Pro Ser Ser Phe Leu Leu Lys Lys Thr Arg  
130 135 140

5 Glu Pro Gly Thr Pro Arg Glu Pro Lys Glu Pro Phe Arg Pro Pro Pro  
145 150 155 160

Ile Thr Pro His Glu Tyr Met Leu Ser Leu Tyr Arg Thr Leu Ser Asp  
165 170 175

Ala Asp Arg Lys Gly Gly Asn Ser Ser Val Lys Leu Glu Ala Gly Leu  
10 180 185 190

Ala Asn Thr Ile Thr Ser Phe Ile Asp Lys Gly Gln Asp Asp Arg Gly  
195 200 205

Pro Ala Val Arg Lys Gln Arg Tyr Val Phe Asp Ile Ser Ala Leu Glu  
210 215 220

15 Lys Asp Gly Leu Leu Gly Ala Glu Leu Arg Ile Leu Arg Lys Lys Pro  
225 230 235 240

Leu Asp Val Ala Lys Pro Ala Val Pro Ser Ser Gly Arg Val Ala Gln  
245 250 255

Leu Lys Leu Ser Ser Cys Pro Ser Gly Arg Gln Pro Ala Ala Leu Leu  
20 260 265 270

Asp Val Arg Ser Val Pro Gly Leu Asp Gly Ser Gly Trp Glu Val Phe  
275 280 285

Asp Ile Trp Lys Leu Phe Arg Asn Phe Lys Asn Ser Ala Gln Leu Cys  
290 295 300

25 Leu Glu Leu Glu Ala Trp Glu Arg Gly Arg Ala Val Asp Leu Arg Gly  
305 310 315 320

Leu Gly Phe Glu Arg Thr Ala Arg Gln Val His Glu Lys Ala Leu Phe  
325 330 335

Leu Val Phe Gly Arg Thr Lys Lys Arg Asp Leu Phe Phe Asn Glu Ile  
30 340 345 350

-44-

Lys Ala Arg Ser Gly Gln Asp Asp Lys Thr Val Tyr Glu Tyr Leu Phe  
355 360 365

Ser Gln Arg Arg Lys Arg Arg Ala Pro Leu Ala Asn Arg Gln Gly Lys  
370 375 380

5 Arg Pro Ser Lys Asn Leu Lys Ala Arg Cys Ser Arg Lys Ala Leu His  
385 390 395 400

Val Asn Phe Lys Asp Met Gly Trp Asp Asp Trp Ile Ile Ala Pro Leu  
405 410 415

10 Glu Tyr Glu Ala Phe His Cys Glu Gly Leu Cys Glu Phe Pro Leu Arg  
420 425 430

Ser His Leu Glu Pro Thr Asn His Ala Val Ile Gln Thr Leu Met Asn  
435 440 445

Ser Met Asp Pro Glu Ser Thr Pro Pro Thr Cys Cys Val Pro Thr Arg  
450 455 460

15 Leu Ser Pro Ile Ser Ile Leu Phe Ile Asp Ser Ala Asn Asn Val Val  
465 470 475 480

Tyr Lys Gln Tyr Glu Asp Met Val Val Glu Ser Cys Gly Cys Arg  
485 490 495

(2) INFORMATION FOR SEQ ID NO:11:

20. (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 124 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:  
(B) CLONE: GDF-1

(ix) FEATURE:  
(A) NAME/KEY: Protein  
30 (B) LOCATION: 1..124

-45-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

	Arg	Leu	Arg	Arg	Arg	His	Thr	Glu	Pro	Arg	Val	Glu	Val	Gly	Pro	Val	Gly
	1					5					10					15	
5	Thr	Cys	Arg	Thr	Arg	Arg	Leu	His	Val	Ser	Phe	Arg	Glu	Val	Gly	Trp	
							20			25					30		
	His	Arg	Trp	Val	Ile	Ala	Pro	Arg	Gly	Phe	Leu	Ala	Asn	Phe	Cys	Gln	
					35				40				45				
10	Gly	Thr	Cys	Ala	Leu	Pro	Glu	Thr	Leu	Arg	Gly	Pro	Gly	Pro	Pro		
					50				55			60					
10	Ala	Leu	Asn	His	Ala	Val	Leu	Arg	Ala	Leu	Met	His	Ala	Ala	Ala	Pro	
					65				70			75			80		
	Thr	Pro	Gly	Ala	Gly	Ser	Pro	Cys	Cys	Val	Pro	Glu	Arg	Leu	Ser	Pro	
						85				90			95				
15	Ile	Ser	Val	Leu	Phe	Phe	Asp	Asn	Glu	Asp	Asn	Val	Val	Leu	Arg	His	
					100				105			110					
	Tyr	Glu	Asp	Met	Val	Val	Asp	Glu	Cys	Gly	Cys	Arg					
					115				120								

(2) INFORMATION FOR SEQ ID NO:12:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 118 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:

- (B) CLONE: GDF-3

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..118

-46-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Arg Lys Arg Arg Ala Ala Ile Ser Val Pro Lys Gly Phe Cys Arg Asn  
1 5 10 15

Phe Cys His Arg His Gln Leu Phe Ile Asn Phe Gln Asp Leu Gly Trp  
5 20 25 30

His Lys Trp Val Ile Ala Pro Lys Gly Phe Met Ala Asn Tyr Cys His  
35 40 45

Gly Glu Cys Pro Phe Ser Met Thr Thr Tyr Leu Asn Ser Ser Asn Tyr  
50 55 60

10 Ala Phe Met Gln Ala Leu Met His Met Ala Asp Pro Lys Val Pro Lys  
65 70 75 80

Ala Val Cys Val Pro Thr Lys Leu Ser Pro Ile Ser Met Leu Tyr Gln  
85 90 95

15 Asp Ser Asp Lys Asn Val Ile Leu Arg His Tyr Glu Asp Met Val Val  
100 105 110

Asp Glu Cys Gly Cys Gly  
115

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:  
20 (A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: GDF-5

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-47-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Leu Ala Asn Arg Gln Gly Lys Arg Pro Ser Lys Asn Leu Lys Ala  
1 5 10 15

Arg Cys Ser Arg Lys Ala Leu His Val Asn Phe Lys Asp Met Gly Trp  
5 20 25 30

Asp Asp Trp Ile Ile Ala Pro Leu Glu Tyr Glu Ala Phe His Cys Glu  
35 40 45

Gly Leu Cys Glu Phe Pro Leu Arg Ser His Leu Glu Pro Thr Asn His  
50 55 60

10 Ala Val Ile Gln Thr Leu Met Asn Ser Met Asp Pro Glu Ser Thr Pro  
65 70 75 80

Pro Thr Cys Cys Val Pro Thr Arg Leu Ser Pro Ile Ser Ile Leu Phe  
85 90 95

Ile Asp Ser Ala Asn Asn Val Val Tyr Lys Gln Tyr Glu Asp Met Val  
15 100 105 110

Val Glu Ser Cys Gly Cys Arg  
115

(2) INFORMATION FOR SEQ ID NO:14:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: GDF-9

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-48-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ser Phe Asn Leu Ser Glu Tyr Phe Lys Gln Phe Leu Phe Pro Gln Asn  
1 5 10 15

Glu Cys Glu Leu His Asp Phe Arg Leu Ser Phe Ser Gln Leu Lys Trp  
5 20 25 30

Asp Asn Trp Ile Val Ala Pro His Arg Tyr Asn Pro Arg Tyr Cys Lys  
35 40 45

Gly Asp Cys Pro Arg Ala Val Arg His Arg Tyr Gly Ser Pro Val His  
50 55 60

10 Thr Met Val Gln Asn Ile Ile Tyr Glu Lys Leu Asp Pro Ser Val Pro  
65 70 75 80

Arg Pro Ser Cys Val Pro Gly Lys Tyr Ser Pro Leu Ser Val Leu Thr  
85 90 95

Ile Glu Pro Asp Gly Ser Ile Ala Tyr Lys Glu Tyr Glu Asp Met Ile  
15 100 105 110

Ala Thr Arg Cys Thr Cys Arg  
115

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 118 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-2

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..118

-49-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Arg Glu Lys Arg Gln Ala Lys His Lys Gln Arg Lys Arg Leu Lys Ser  
1 5 10 15

5 Ser Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp  
20 25 30

Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His  
35 40 45

Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His  
50 55 60

10 Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys  
65 70 75 80

Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu  
85 90 95

15 Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val  
100 105 110

Glu Gly Cys Gly Cys Arg  
115

(2) INFORMATION FOR SEQ ID NO:16:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 118 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-4

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..118

-50-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys  
1 5 10 15

Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp  
5 20 25 30

Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His  
35 40 45

Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His  
50 55 60

10 Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys  
65 70 75 80

Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu  
85 90 95

15 Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val  
100 105 110

Glu Gly Cys Gly Cys Arg  
115

(2) INFORMATION FOR SEQ ID NO:17:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Vgr-1

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-51-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ser Arg Gly Ser Gly Ser Ser Asp Tyr Asn Gly Ser Glu Leu Lys Thr  
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp  
5 20 25 30

Gln Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp  
35 40 45

Gly Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His  
50 55 60

Ala Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro  
10 65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr  
85 90 95

Phe Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val  
15 100 105 110

Val Arg Ala Cys Gly Cys His  
115

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:  
20 (A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: OP-1

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-52-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser Asp Gln Arg Gln  
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp  
5 20 25 30

Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu  
35 40 45

Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr Asn His  
50 55 60

10 Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr Val Pro  
65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr  
85 90 95

15 Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val  
100 105 110

Val Arg Ala Cys Gly Cys His  
115

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 119 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-5

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..119

-53-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ser Arg Met Ser Ser Val Gly Asp Tyr Asn Thr Ser Glu Gln Lys Gln  
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp  
5 20 25 30

Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp  
35 40 45

Gly Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His  
50 55 60

10 Ala Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro  
65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr  
85 90 95

15 Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val  
100 105 110

Val Arg Ser Cys Gly Cys His  
115

(2) INFORMATION FOR SEQ ID NO:20:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 120 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: BMP-3

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..120

-54-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Glu Gln Thr Leu Lys Lys Ala Arg Arg Lys Gln Trp Ile Glu Pro Arg  
1 5 10 15

Asn Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp  
5 20 25 30

Ser Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser  
35 40 45

Gly Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His  
50 55 60

10 Ala Thr Ile Gln Ser Ile Val Arg Ala Val Gly Val Val Pro Gly Ile  
65 70 75 80

Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu  
85 90 95

15 Phe Phe Asp Glu Asn Asn Val Val Leu Lys Val Tyr Pro Asn Met  
100 105 110

Thr Val Glu Ser Cys Ala Cys Arg  
115 120

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:  
20 (A) LENGTH: 116 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: MIS

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..116

-55-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Pro Gly Arg Ala Gln Arg Ser Ala Gly Ala Thr Ala Ala Asp Gly  
1 5 10 15

Pro Cys Ala Leu Arg Glu Leu Ser Val Asp Leu Arg Ala Glu Arg Ser  
5 20 25 30

Val Leu Ile Pro Glu Thr Tyr Gln Ala Asn Asn Cys Gln Gly Val Cys  
35 40 45

Gly Trp Pro Gln Ser Asp Arg Asn Pro Arg Tyr Gly Asn His Val Val  
50 55 60

10 Leu Leu Leu Lys Met Gln Ala Arg Gly Ala Ala Leu Ala Arg Pro Pro  
65 70 75 80

Cys Cys Val Pro Thr Ala Tyr Ala Gly Lys Leu Leu Ile Ser Leu Ser  
85 90 95

15 Glu Glu Arg Ile Ser Ala His His Val Pro Asn Met Val Ala Thr Glu  
100 105 110

Cys Gly Cys Arg  
115

(2) INFORMATION FOR SEQ ID NO:22:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 122 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Inhibit-alpha

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..122

-56-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ala Leu Arg Leu Leu Gln Arg Pro Pro Glu Glu Pro Ala Ala His Ala  
1 5 10 15

Asn Cys His Arg Val Ala Leu Asn Ile Ser Phe Gln Glu Leu Gly Trp  
5 20 25 30

Glu Arg Trp Ile Val Tyr Pro Pro Ser Phe Ile Phe His Tyr Cys His  
35 40 45

Gly Gly Cys Gly Leu His Ile Pro Pro Asn Leu Ser Leu Pro Val Pro  
50 55 60

10 Gly Ala Pro Pro Thr Pro Ala Gln Pro Tyr Ser Leu Leu Pro Gly Ala  
65 70 75 80

Gln Pro Cys Cys Ala Ala Leu Pro Gly Thr Met Arg Pro Leu His Val  
85 90 95

15 Arg Thr Thr Ser Asp Gly Gly Tyr Ser Phe Lys Tyr Glu Thr Val Pro  
100 105 110

Asn Leu Leu Thr Gln His Cys Ala Cys Ile  
115 120

(2) INFORMATION FOR SEQ ID NO:23:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 122 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Inhibin-beta-alpha

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..122

-57-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

His Arg Arg Arg Arg Arg Gly Leu Glu Cys Asp Gly Lys Val Asn Ile  
1 5 10 15

5 Cys Cys Lys Lys Gln Phe Phe Val Ser Phe Lys Asp Ile Gly Trp Asn  
20 25 30

Asp Trp Ile Ile Ala Pro Ser Gly Tyr His Ala Asn Tyr Cys Glu Gly  
35 40 45

Glu Cys Pro Ser His Ile Ala Gly Thr Ser Gly Ser Ser Leu Ser Phe  
50 55 60

10 His Ser Thr Val Ile Asn His Tyr Arg Met Arg Gly His Ser Pro Phe  
65 70 75 80

Ala Asn Leu Lys Ser Cys Cys Val Pro Thr Lys Leu Arg Pro Met Ser  
85 90 95

15 Met Leu Tyr Tyr Asp Asp Gly Gln Asn Ile Ile Lys Lys Asp Ile Gln  
100 105 110

Asn Met Ile Val Glu Glu Cys Gly Cys Ser  
115 120

(2) INFORMATION FOR SEQ ID NO:24:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 121 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: Inhibin-beta-beta

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..121

-58-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

His Arg Ile Arg Lys Arg Gly Leu Glu Cys Asp Gly Arg Thr Asn Leu  
1 5 10 15

5 Cys Cys Arg Gln Gln Phe Phe Ile Asp Phe Arg Leu Ile Gly Trp Asn  
20 25 30

Asp Trp Ile Ile Ala Pro Thr Gly Tyr Tyr Gly Asn Tyr Cys Glu Gly  
35 40 45

Ser Cys Pro Ala Tyr Leu Ala Gly Val Pro Gly Ser Ala Ser Ser Phe  
50 55 60

10 His Thr Ala Val Val Asn Gln Tyr Arg Met Arg Gly Leu Asn Pro Gly  
65 70 75 80

Thr Val Asn Ser Cys Cys Ile Pro Thr Lys Leu Ser Thr Met Ser Met  
85 90 95

15 Leu Tyr Phe Asp Asp Glu Tyr Asn Ile Val Lys Arg Asp Val Pro Asn  
100 105 110

Met Ile Val Glu Glu Cys Gly Cys Ala  
115 120

(2) INFORMATION FOR SEQ ID NO:25:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 115 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: TGF-beta-1

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..115

-59-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

His Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser Thr Glu Lys  
1 5 10 15

Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gly  
5 20 25 30

Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Leu  
35 40 45

Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr Ser Lys Val  
50 55 60

Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys  
10 65 70 75 80

Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly  
85 90 95

Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val Arg Ser Cys  
15 100 105 110

Lys Cys Ser  
115

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 115 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: TGF-beta-2

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..115

-60-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Lys Lys Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp  
1 5 10 15

Asn Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly  
5 20 25 30

Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ala  
35 40 45

Gly Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His Ser Arg Val  
50 55 60

10 Leu Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys  
65 70 75 80

Cys Val Ser Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly  
85 90 95

15 Lys Thr Pro Lys Ile Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys  
100 105 110

Lys Cys Ser  
115

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
20 (A) LENGTH: 115 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (vii) IMMEDIATE SOURCE:  
(B) CLONE: TGF-beta-3

(ix) FEATURE:  
(A) NAME/KEY: Protein  
(B) LOCATION: 1..115

-61-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

-62-

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following  
5 claims.

**CLAIMS**

1. Substantially pure growth differentiation factor-5 (GDF-5) and functional fragments thereof.
2. An isolated polynucleotide sequence encoding the GDF-5 polypeptide of claim 1.
3. The polynucleotide sequence of claim 2, wherein the polynucleotide is isolated from a mammalian cell.
4. The polynucleotide of claim 3, wherein the mammalian cell is selected from the group consisting of mouse, rat, and human cell.
5. An expression vector including the polynucleotide of claim 2.
6. The vector of claim 5, wherein the vector is a plasmid.
7. The vector of claim 5, wherein the vector is a virus.
8. A host cell stably transformed with the vector of claim 5.
9. The host cell of claim 8, wherein the cell is prokaryotic.
10. The host cell of claim 8, wherein the cell is eukaryotic.
11. Antibodies reactive with the polypeptide of claim 1 or fragments thereof.
12. The antibodies of claim 11, wherein the antibodies are polyclonal.

-64-

13. The antibodies of claim 11, wherein the antibodies are monoclonal.
14. A method of detecting a cell proliferative disorder comprising contacting the antibody of claim 11 with a specimen of a subject suspected of having a GDF-5 associated disorder and detecting binding of the antibody.
15. The method of claim 14, wherein the cell proliferative disorder is a uterine neoplasm or endometriosis.
16. The method of claim 14, wherein the cell proliferative disorder is a skeletal disorder.
17. The method of claim 14, wherein the detecting is *in vivo*.
18. The method of claim 17, wherein the antibody is detectably labeled.
19. The method of claim 18, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.
20. The method of claim 14, wherein the detection is *in vitro*.
21. The method of claim 20, wherein the antibody is detectably labeled.
22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemoluminescent compound and an enzyme.

-65-

23. A method of treating a cell proliferative disorder associated with expression of GDF-5, comprising contacting the cells with a reagent which suppresses the GDF-5 activity.
24. The method of claim 23, wherein the reagent is an anti-GDF-5 antibody.
25. The method of claim 23, wherein the reagent is a GDF-5 antisense sequence.
26. The method of claim 23, wherein the cell proliferative disorder is a uterine neoplasm or endometriosis.
27. The method of claim 23, wherein the cell proliferative disorder is a skeletal disorder.
28. The method of claim 23, wherein the reagent which suppresses GDF-5 activity is introduced to a cell using a vector.
29. The method of claim 28, wherein the vector is a colloidal dispersion system.
30. The method of claim 29, wherein the colloidal dispersion system is a liposome.
31. The method of claim 30, wherein the liposome is essentially target specific.
32. The method of claim 31, wherein the liposome is anatomically targeted.

-66-

33. The method of claim 31, wherein the liposome is mechanistically targeted.
34. The method of claim 33, wherein the mechanistic targeting is passive.
35. The method of claim 33, wherein the mechanistic targeting is active.
36. The method of claim 35, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.
37. The method of claim 36, wherein the protein moiety is an antibody.
38. The method of claim 37, wherein the vector is a virus.
39. The method of claim 38, wherein the virus is an RNA virus.
40. The method of claim 39, wherein the RNA virus is a retrovirus.
41. The method of claim 40, wherein the retrovirus is essentially target specific.

**a**

10.5 placenta  
testis  
seminal vesicle  
ovary  
oviduct  
uterus  
brain  
thymus  
heart  
lung  
kidney  
adrenal  
spleen  
liver  
intestine  
pancreas

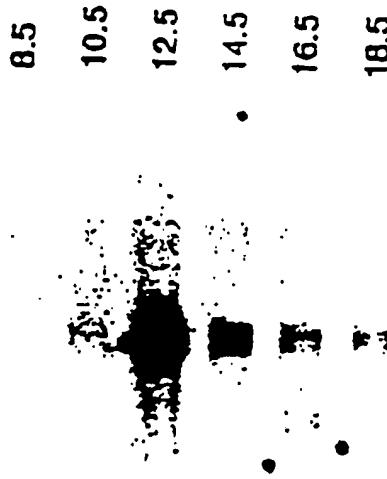
**b**

FIGURE 1

2/7

FIGURE 2

Figure 3

GDF-1 ✓	RLLRHTPRVEVCFVCTDTRRIKVSF-REVGWNRWVIAPROFANTIOCTALPFTKUHCG??
GDF-3 ✓	KKRRAAISVPKGCFRNFHRHQSFDF-CDLGWHRKAVIAKCGFMANHCGEDFSGTYTNG---
GDF-5 ✓	PLANKOGKRPKSNLKAPESTKALKVNFK-KEMGHDWVIAPELEYAFHOCGIEFFP-RSHLSD---
GDF-9	STNLSTYTKQPLFTGNEEELHDYALSP-SGLXWUMWIVAPHRYNPRYKCGPGRVTRERYCS---
~	
BMP-2 ✓	EDGRCQANGHQKQKRLKSSKRMPLYDF-SDVGWNRHIVAPPGYHAFYHC2DFPFLADHLS---
BMP-4 ✓	KRSKHKHSCRARKKMKRKRHSLSYDF-SDVGWNRHIVAPPGYHAFYHC2DFPFLADHLS---
Vgr-1 ✓	SRGSCSSDYNCSZLTTACKKHELYVSF-CDIAGQDWTIAPEGYAAVYCEGACDFPFLADHLS---
OP-1 ✓	LRKANVAEKSSSDQRQACDKKHELYVSF-CDIAGQDWTIAPEGYAAVYCEGACDFPFLADHLS---
BMP-5 ✓	ERKSSJVDYNTTSEKQACDKKHELYVSF-CDIAGQDWTIAPEGYAAVYCEGACDFPFLADHLS---
BMP-3 ✓	EDTIAKARRKQWIEPRNQDARYLKVDF-ADIGNSWITIISPKS7PDAYLSCDQDFPMPKSLXPS-
MIS	CPCRAORSAGATADGFDALRSTSVD1---RAGL2TLP-DETYQAHNSDQWVWQSDRNPRY--
Inhibin α	ALRLLORPPEEPAAHANCHRVALNIST-QELGWERWIVYPPSF17RDKKTTTLLX17PHLCPV-
Inhibin βA	EDRRLRAGLCEGDKV-NLILKQWVY5F-KDQGKNTIAPSGTQHANVQDCECPKSHACTSCS5F-
Inhibin βB	PRTRKRCLEDCDT-NLILRQGFTDF-KLIGANQNTIAPGTYQHNGCSCDPAYLACVPGSAS-
TGF-β1 ✓	HRRLDNTNYCYSSTEKEVWQYDIFRKDLSK-WIETKQYHANHDLGCPYFWSLW-----
TGF-β2 ✓	KKRALLDAAVCFRNVQDNCLRFLYIDFKRDLGK-WIHEPKGYNANFACXEPYLWEED.-----
TGF-β3 ✓	KKRALLDAAVCFRNVQDNCLRFLYIDFKRDLGK-WIHEPKGYNANFACXEPYLWEED.-----
~	
GDF-1	ALNHAVLRALYHA-AAPTPGAGSPTC1--PTELS?ISV17P-DNECDNYYIURYEDMVVDCDOR
GDF-3	-SNYALFMCALDQH---ADPKVPKAVCV--PTKLS?ISMLYC-DSDRKNTLRYHEDMVVDCDOR
GDF-5	-TMAHATVGTLMNS--MDYESTPPTICW--PTKLS?ISL1?I-DSANNNVYKCYEDMVVDCDOR
GDF-9	-DVKTDKVNQIYI--EEDPSVPRPSCV--PCKTSPLSVLTI-EPDGSIAKKEYEDMIAATCQH
~	
BMP-2	-TMAHATVOTLVNS--VNSKIPKACCV--PTKLSAISMLYL-CZENKVVLLQYQDMVVECDOR
BMP-4	-TMAHATVOTLVNS---VNSS2PKACCV--PTKLSAISMLYL-CZEDKVVLLQYQDMVVECDOR
Vgr-1	-TMAHATVOTLVNL--KNPZEVVPKRCGA--PTKLSAISVLYP-CDSNTLQCYRANMVVRACDOR
OP-1	-TMAHATVOTLVHF--INPETVPKRCGA--PTKLSAISVLYP-CDSNTLQCYRANMVVRACDOR
BMP-5	-TMAHATVOTLVHL--KFPDQVVPKRCGA--PTKLSAISVLYF-CDSNTLQCYRANMVVRACDOR
BMP-3	--MKTATQSTVRA-VCVTPC:2EPCDV--PDKQSCSLT1PF-CENKVVLLQYQDMVVECDOR
MIS	-CNEVVLLKQCA--RCAALARPFCOV--PTIYMTKLLISLSEK--ISAEHVPMVVAECDOR
Inhibin α	-PGAPPTPAQPS---LLPGCAHEDALPCTQIRPLAVRPTSDGYSFKYET/7NLLTQHCKC
Inhibin βA	-SPHSTVQHRYRCHSPPFANLQSCV--PTKLSRPHMSMLYY-DCGQNI-EXCTQHMIIVECQG
Inhibin βB	-S7TITIVVNGYRMRCLNCTVNSCCT--PTKLS?ISMLYF-CUQYNTIVYRQDVPAKIVVECQG
TGF-β1	-TOYSKVLALYNO--KNPCLSAAPTCV--PQALE2PL2VYV-VGRTPKV-EQLSNQIVRSOKL
TGF-β2	-TQHSRVLSLYNT--INPEASASPCOV--SGDL2PL2VYV-ICXTPRI-EQLSNQIVXZOKS
TGF-β3	-TQKSTVLGLYNT--INPEASASPCOV--PQJL8PL2VYV-VGRTPKV-EQLSNQIVXZOKS

FIGURE 3a

371	395
GDF-5 R R K R R A P L A N R O C K R P S - - - - -	- - - - - K N L K A R C S
GDF-6 R R R R R T A P A S R H C K R H G - - - - -	- - - - - K K S R L R C S
GDF-7 R R R R R T A L A G T R C A Q C S G A G R G H G R R G R S R C S	
<hr/>	
396	446
GDF-5 R K A L H V N P K D H G W D D W I I A P L E Y E A F H C E G L C E F P L R S H L E P T N H A V I Q T L	
GDF-6 R K P L H V N P K E L G W D D W I I A P L E Y E A Y H C E G V C D F P L R S H L E P T N H A I I Q T L	
GDF-7 R K S L H V D P K E L G W D D W I I A P L D Y E A Y H C E G V C D F P L R S H L E P T N H A I I Q T L	
<hr/>	
447	495
GDF-5 M N S H M D P E S T P P T C C V P T R L S P I S I L F I D S A N N V V Y K Q Y E D M V V E S C G C R	
GDF-6 M N S H M D P G S T P P S C C V P T K L T P I S I L Y I D A G N N V V Y K Q Y E D M V V E S C G C R	
GDF-7 L N S M A P D A A P A S C C V P A R L S P I S I L Y I D A A N N V V Y K Q Y E D M V V E A C G C R	

FIGURE 3b

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30			
GDP-1	100	33	50	46	44	48	35	27	42	43	46	47	46	42	34	21	37	35	33	32	33												
GDP-2	-	100	42	67	51	48	31	32	52	51	55	52	53	54	29	20	32	25	26	28	30												
GDP-3	-	-	100	49	49	46	41	33	53	50	53	50	53	52	42	22	25	42	42	36	31	32											
GDP-5	-	-	-	-	-	-	-	37	33	67	57	51	51	52	47	27	26	40	37	33	34	37											
GDP-6	-	-	-	-	-	-	-	38	34	57	56	53	63	61	46	26	27	43	39	35	36	38											
GDP-7	-	-	-	-	-	-	-	37	33	67	57	52	53	52	46	25	26	41	36	36	36	38											
GDP-8	-	-	-	-	-	-	-	-	100	27	41	38	45	42	42	38	3-	26	38	42	36	37	37										
GDP-9	-	-	-	-	-	-	-	-	-	100	33	34	31	30	3-	29	21	27	30	31	23	26	25										
BMP-2	-	-	-	-	-	-	-	-	-	-	61	60	61	60	46	27	22	42	42	36	34	36											
BMP-4	-	-	-	-	-	-	-	-	-	-	60	58	59	67	27	22	41	42	34	33	35												
Vgr-1	-	-	-	-	-	-	-	-	-	-	-	-	-	44	24	25	44	41	35	37	39												
OP-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	42	27	24	43	42	34	38	38											
BMP-5	-	-	-	-	-	-	-	-	-	-	-	-	-	43	24	24	43	37	34	35	36												
BMP-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	30	29	36	37	32	32	32											
ATB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	18	24	25	28	23	25										
Inhibin $\alpha$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	26	25	23	22	24									
Inhibin $\beta A$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	41	37	36											
TGF- $\beta 1$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
TGF- $\beta 2$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
TGF- $\beta 3$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					

FIGURE 4

FIGURE 5a



FIGURE 5b



FIGURE 5c



FIGURE 5d



FIGURE 5e

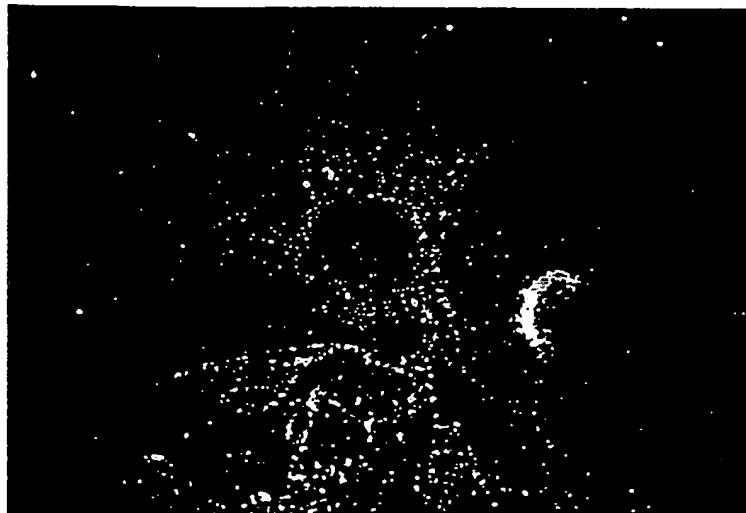
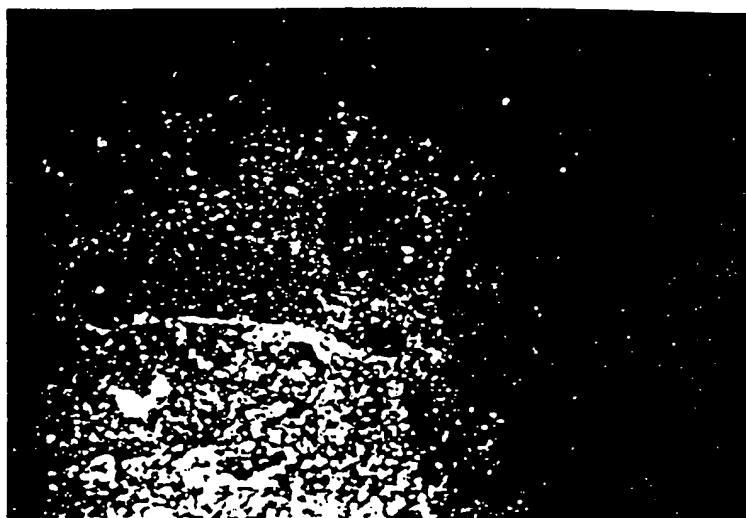


FIGURE 5f



## INTERNATIONAL SEARCH REPORT

Int. application No.  
PCT/US94/00657

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Molecular Endocrinology, Vol. 4, issued 1990, Lee, S.-J., "Identification Of A Novel Member (GDF-1) Of The Transforming Growth Factor- $\beta$ Superfamily", pages 1034-1040, see entire document.	2-6, 8, 9
-		_____
Y	Proc. Natl. Acad. Sci., USA, Vol. 88, Issued May 1991, Lee, S.-J., "Expression Of Growth/Differentiation Factor 1 In The Nervous System: Conservation Of A Bicistronic Structure", pages 4250-4254, see entire document.	1-13
Y	Meth. Enzymol., Vol. 100, issued 1983, Beltz et al., "Isolation of Multigene Families And Determination Of Homologies By Filter Hybridization Methods", pages 266-285, see entire document.	1-13

 Further documents are listed in the continuation of Box C.

See patent family annex.

•	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
•A	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
•E	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
•L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
•O	document referring to an oral disclosure, use, exhibition or other means		
•P	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

05 APRIL 1994

Date of mailing of the international search report

APR 15 1994

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Authorized officer

CHRISTOPHER S. F. LOW

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

Int. application No.  
PCT/US94/00657

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Sambrook et al., "Molecular Cloning, A Laboratory Manual", Second Edition, published 1989 by Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pages 11.2-11.11, 11.17-11.19, see all cited pages.	1-13
Y	US, A, 4,675,285 (CLARK et al.) 23 June 1987, see entire document.	1-13

**INTERNATIONAL SEARCH REPORT**Int'l application No.  
PCT/US94/00657**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:  
1-13
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/00657

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

C07K 3/00, 13/00, 15/28, 17/00; C07H 15/12, 17/00; C12N 15/70, 15/79, 15/00, 7/00, 1/20, 5/16, 1/21

## A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

530 / 350, 399, 387.1; 536 / 23.1, 23.51; 435 / 320.1, 235.1, 252.3, 240.2, 172.3

## B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

530 / 350, 399, 387.1; 536 / 23.1, 23.51; 435 / 320.1, 235.1, 252.3, 240.2, 172.3

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

Automated Patent System - USPAT, JPOABS

Dialog one search files: 5, 265, 266

EMBL-NEW, GenBank 80, GenBank-NEW, N-GeneSeq 13, UEMBL 37\_80, A-GeneSeq 13, PIR 38, and Swiss-Prot 27

Search terms: growth, differentiation, factor 5, vector, viral, virus, plasmid

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This international application contains the following inventions or groups of inventions which are not so linked by the identical special technical feature so as to form a single inventive concept under the criteria of PCT Rule 13.2.

I. Claims 1, drawn to growth differentiation factor 5 (GDF-5) are for example, classified in Class 530, subclasses 350 and 399.

II. Claims 2-10, drawn to polynucleotides encoding growth differentiation factor 5 as well as vectors and host cells containing same are for example, classified in Class 536, subclass 23.1 and 23.51 and Class 435 subclasses 320.1, 235.1, 252.3, and 240.2.

III. Claims 11-13, drawn to antibodies to growth differentiation factor 5 are for example, classified in at least Class 530, subclass 387.1.

IV. Claims 14-22, drawn to a method of detecting a cell proliferative disorder by binding an antibody to the sample specimen are for example, classified in Class 435, subclass 7.1.

V. Claims 23-41, drawn to a method of treating a cell proliferative disorder by binding an antibody to the sample specimen are for example, classified in at least Class 424, subclass 85.8. Note that in Group IV, claim 14 is generic to a plurality of recited species which are: species (A) a neoplasm or (B) endometriosis (claim 15); species (C) a skeletal disorder (claim 16) determined by in vivo measurement (claim 17) using labeled (claim 18) compounds which are (claim 19) radioisotopes (Class 435, subclass 504); species (D) a skeletal disorder (claim 16) determined by in vivo measurement (claim 17) using labeled (claim 18) compounds which are (claim 19) luminescent compounds (Class 435, subclass 8); species (E) where the measurement is in vitro (claim 20) and the antibody is labeled (claim 21) with radioisotopes (Class 436, subclass 504); species (F) where the measurement is in vitro (claim 20) and the antibody is labeled (claim 21) with luminescent compounds such as in Class 435, subclass 8. Species A of Group IV will be examined with Group IV should applicant pay the additional fee for searching Group IV where species B through F constitute five additional species which will be searched upon payment of the requisite additional fees for each species.

In Group V, claim 23 is generic to a plurality of recited species of reagent and cell proliferative disorder which consist of species (A, claim 24) anti-GDF-5 antibody (Class 424, subclass 85.8); species (B, claim 25) GDF-5 antisense polynucleotide (Class 536, subclass 23.1); species (C, claim 28) where the reagent is a vector which is a colloidal dispersion (claim 29) is a liposome (claim 30) which is targeted (claims 31-37); species (D, claim 28) where the reagent

**INTERNATIONAL SEARCH REPORT**

International application No. PCT/US94/00657
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is a vector which is a (claims 38-41) virus (Class 435, subclass 235.1); species (E) wherein the disorder is a neoplasm or (F) endometriosis (claim 26); species (G) wherein the disorder is a skeletal disorder (claim 27). Species A of Group V will be examined with Group IV should applicant pay the additional fee for searching Group IV where species B through G constitute six additional species which will be searched upon payment of the requisite additional fees for each species.

## INDEX TO JOINT APPENDIX

<u>DOCUMENT</u>	<u>PAGE(S)</u>
Order and Judgment .....	A0001-A0002
Memorandum Opinion dated July 28, 2006. ....	A0003-A0037
Civil Docket in Case No. 1:05-cv-00691 LMB/TRJ .....	A0038-A0046
Excerpts of Plaintiff's Memorandum in Support of Plaintiff's Motion for Summary Judgment.....	A0078, A0093-A0095
U.S. Patent No. 6,899,690 B2.....	A0107-A0124
U.S. Patent No. 6,506,174 B1 .....	A0151-A0167
Document numbered CR000786 .....	A0244
Deposition Exhibit R-4 from the Deposition of Charles F. Reinholtz, Ph.D.....	A0246
Excerpts from the Deposition of Charles F. Reinholtz, Ph.D, October 28, 2005.....	A0251, A0254, A0257, A0266-A0267, A0286, A0294
Excerpts from the Deposition of Ara Boghosian, October 5, 2005.....	A0310-A0311
Excerpts from the Deposition of John Spadavecchia, October 6, 2005.....	A0314, A0316, A0318-A0319
Excerpts from the Declaration of Robin Saunders Ryan, July 25, 2005 .....	A0348-A0351, A0353
Letter from Karl Schwappach to Christian Hunt dated January 26, 2004.....	A0380-A0382
Declaration of Dr. William K . Durfee dated November 18, 2005 and excerpts of attached Expert Report of Dr. William K. Durfee .....	A0385-A0418, A0421, A0423, A0462-A0472

Excerpts from Plaintiff's Memorandum in Opposition to Defendants' Motion for Summary Judgment dated December 1, 2005 .....	A0594, A0609-A0613, A0618-A0619, A0620
Excerpts from Second Declaration of Dr. William K. Durfee, dated December 2, 2005 .....	A0663-A0664
Excerpts from Memorandum of Points and Authorities in Support of Defendants Comfortrac, Inc.'s and Care Rehab and Orthopaedic Products, Inc.'s Motion for Summary Judgment of Non-Infringement dated November 21, 2005 .....	A0706, A0709
Excerpts from Deposition of Duane Saunders, dated September 26, 2005.....	A0783-A0785, A0790-A0792
Excerpts from Defendants' Memorandum of Points and Authorities in Opposition to Plaintiff's Motion for Summary Judgment.....	A0878-A0879
Excerpts from the Expert Report of Charles F. Reinholtz, Ph.D. dated September 21, 2005 .....	A0951-A0952
Excerpts of Plaintiff The Saunders Group, Inc.'s Initial Disclosure of Asserted Claims and Infringement Contentions dated August 26, 2005 .....	A1020-A1068
Excerpts from Rebuttal Report of Charles F. Reinholtz, Ph.D. dated October 14, 2005.....	A1148-A1149
Excerpt from Defendants' Opposition to Plaintiff's Motion for Preliminary Injunction dated August 15, 2005.....	A1288
Excerpts from the Declaration of Ara Boghosian dated August 15, 2005 .....	A1312-A1313
Excerpt from the Declaration of Christian Hunt dated August 15, 2005 .....	A1316
Excerpts from the File History of Application No. 08/334,189, filed November 3, 1994.....	A1469

Excerpts from Application filed November 3, 1994 .....	A1477, A1484-A1488
Office Action dated April 26, 1996.....	A1502-A1510
Applicants' Amendment and Response dated August 14, 1996 .....	A1515-A1523
Office Action dated November 6, 1996 .....	A1526-A1534
Excerpt from Applicants' Amendment and Response dated January 20, 1997 .....	A1536
Excerpts from the File History of U.S. Patent No. 6,506,174 B1 dated January 14, 2003 .....	A1549
Excerpts from Application filed April 30, 1997 .....	A1573-A1574, A1584-A1588
Declaration of William Freed under 37 C.F.R. §1.132 dated April 30, 1997.....	A1599-A1600
Excerpts from Applicant's Preliminary Amendment dated April 30, 1997 .....	A1685-A1686
Office Action dated September 22, 1998 .....	A1705-A1712
Applicants' Amendment and Response dated January 28, 1999.....	A1718-A1726
Second Declaration of H. Duane Saunders under 37 C.F.R. §1.132 dated January 28, 1999 .....	A1727-A1729
Office Action dated March 6, 1999 .....	A1742-A1754
Applicants' Notice of Appeal dated June 9, 1999.....	A1758
Appellants' Brief before the Board of Patent Appeals and Interferences dated October 8, 1999.....	A1765-A1780

Decision on Appeal dated September 26, 2001 .....	A1791-A1810
U.S. Patent 6,506,174 B1 dated January 14, 2003 .....	A1906-A1922
Excerpts from the File History of U.S. Patent No. 6,899,690 B2, dated May 31, 2005 .....	A1950-A2088
U.S. Patent 3,937,216 dated Feb. 10, 1976 (Brown).....	A2272-A2275
U.S. Patent 4,428,276 dated Jan. 31, 1984 (Loveless).....	A2322-A2327
U.S. Patent 5,092,322 dated Mar. 3, 1992 (Gantz).....	A2460-A2470
Excerpts from the Court Discovery Plan Order.....	A2617
Defendants Comfortrac, Inc. and Care Rehab and Orthopaedic Products, Inc.'s Supplemental Preliminary Invalidity Contentions.....	A2620-A2631

UNITED STATES DISTRICT COURT  
CENTRAL DISTRICT OF CALIFORNIA  
WESTERN DIVISION

**K-SWISS V. TARGET CORPORATION**

Case No. CV 06-2314 RJK (RCx)

**INDEX OF DOCUMENTS PRODUCED BY K-SWISS**

	Bates - Begin	Bates - End	Description
	KSW014-000001	KSW014-000380	K-SWISS, Inc.'s Account List: Historical
	KSW014-000381	KSW014-000682	K-SWISS, Inc.'s Account List: Historical
	KSW014-000683	KSW014-000988	K-SWISS, Inc.'s Account List: Historical
	KSW014-000989	KSW014-001297	K-SWISS, Inc.'s Account List: Historical
	KSW014-001298	KSW014-001459	K-SWISS, Inc.'s Account List: Historical
	KSW014-001460	KSW014-001797	PLEADINGS IN K-SWISS, Inc. V. USA Aisiqi Shoes, Inc.
	KSW014-001798	KSW014-002142	K-SWISS, Inc. Annual reports for years 1990, 1993, Instore Purchasing Catalogs, and pleadings in the USA Aisiqi Shoes, Inc. Matter
	KSW014-002143	KSW014-002448	PLEADINGS IN K-SWISS, Inc. V. USA Aisiqi Shoes, Inc.
	KSW014-002449	KSW014-002460	Plaintiff's (K-SWISS, Inc.) First Set of Interrogatories to Defendant USA Aisiqi Shoes, Inc.
	KSW014-002461	KSW014-002462	Defendant USA Aisiqi Shoes, Inc.'s Notice of Production of Documents pursuant to K-SWISS, Inc.'s First set of Requests for Documents
	KSW014-002463	KSW014-002503	Defendant USA Aisiqi Shoes, Inc.'s Objections and Responses to K-SWISS, Inc.'s First Set of Requests for Documents
	KSW014-002504	KSW014-002520	Defendant USA Aisiqi Shoes, Inc.'s Objections and Responses to K-SWISS, Inc.'s First Set of Interrogatories
	KSW014-002521	KSW014-002524	Defendants Initial Disclosures

	Bates - Begin	Bates - End	Description
	KSW014-002525	KSW014-002525	Order granting Plaintiff's Motion to Dismiss Counterclaim for lack of subject matter jurisdiction
	KSW014-002526	KSW014-002528	Plaintiff's Notice of Motion and Motion to Dismiss Counterclaim for Lack of Subject Matter Jurisdiction
	KSW014-002953	KSW014-002996	K-SWISS, Inc.' Footwear / Apparel Catalog: Third Quarter 2001
	KSW014-002997	KSW014-003044	K-SWISS, Inc.' Footwear / Apparel Catalog: Second Quarter 2002
	KSW014-003045	KSW014-003085	K-SWISS, Inc.' Footwear / Apparel Catalog: Third Quarter 2002
	KSW014-003086	KSW014-003097	K-SWISS, Inc.' Footwear / Apparel Catalog: Fourth Quarter 2002
	KSW014-003098	KSW014-003132	K-SWISS, Inc. USA's Retail Accounts List

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